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Multiple Diffusion Indices Reveals White Matter Degeneration in Alzheimer's Disease and Mild Cognitive Impairment: A Tract-Based Spatial Statistics Study

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Abstract. Alzheimer's disease (AD) is a progressive neurodegenerative disease involving the decline of memory and other cognitive functions. Mild cognitive impairment (MCI) represents a transition phase between normal aging and early AD. The degeneration patterns of the white matter across the brain in AD and MCI remain largely unclear. Here we used diffusion tensor imaging and tract-based spatial statistics (TBSS) to investigate white matter changes in multiple diffusion indices (e.g., fractional anisotropy, axial, radial and mean diffusivities) in both AD and MCI patients. Compared with the normal controls, the AD patients had reduced fractional anisotropy and increased axial, radial and mean diffusivities in widespread white matter structures, including the corpus callosum and the white matter of lateral temporal cortex, the posterior cingulate cortex/precuneus and the fronto-parietal regions. Similar white matter regions with reduced anisotropy were also found in MCI patients but with a much less extent than in AD. Between the AD and MCI groups, there were significant differences in the axial and mean diffusivities of the white matter tracts adjacent to the posterior cingulate cortex/precuneus without anisotropy changes. Taken together, our findings based upon multiple diffusion indices (FA, axial, radial and mean diffusivities) suggest distinct degeneration behaviors of the white matter in AD and MCI.

Keywords: Connectivity, axial diffusivity, radial diffusivity, DTI, TBSS

INTRODUCTION

Alzheimer's disease (AD) is the most common degenerative dementia in the elderly and is characterized as a progressive neurodegenerative disease involving the decline of memory and other cognitive functions [1]. Mild cognitive impairment (MCI) is defined as memory impairment in the setting of normal general cognitive function without dementia [2]. In particular, the amnestic subtype of MCI is considered

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as a transition phase between normal aging and AD [2]. In the past decade, advanced magnetic resonance imaging (MRI) approaches have been extensively used for the assessment of brain structural and functional alterations in patients with AD and MCI [3–7], which are important for our understanding the neuropathological mechanisms of the diseases.

Diffusion tensor imaging is an advanced MRI technique for evaluating the white matter integrity and anatomical connectivity *in vivo* [8, 9], and has been increasingly employed to investigate the diffusion changes of the white matter in AD and MCI (for reviews, see [10, 11]). Fractional anisotropy (FA) and mean diffusivity (MD) are two important diffusion metrics for the analysis of DTI data: the former reflects the degree of directionality of cellular structures (i.e., structural integrity) within the fiber tracts by measuring anisotropic water diffusion [12, 13], while the latter measures diffusion in the noncolinear direction or free diffusion [13]. To date, these two diffusion metrics have been widely used to assess white matter changes in brain diseases [14–16]; however, it should be noted that they are not sufficient to reflect pathological changes in white matter at a microstructural level [17, 18]. Recently, two other metrics of water movement, parallel (axial diffusivity, λ_1) and perpendicular (radial diffusivity, λ_{23}) to the primary diffusion direction have been proposed to capture the neural bases of diffusion changes in white matter tracts [5, 17, 19–21]. Studies including histological verification have put forth the notion that alterations in axial and radial diffusivities may reflect specific changes in the axon and myelin, respectively [22, 23].

The vast majority of previous DTI studies in AD and MCI patients have concentrated on FA and/or MD changes without consideration of the component eigenvalues [10]. Notably, there is a lack of empirical biological evidence that FA changes necessarily capture the full extent of white matter changes in AD and MCI patients. By using multiple diffusion indices (FA, MD, λ_1 and λ_{23}), Huang et al. investigated MCI- and AD-related changes exclusively in the white matter of the temporal lobe [24]. More recently, Acosta-Cabronero et al. utilized these diffusion indices to examine white matter degeneration in the whole brain in early AD patients and found that the use of diffusivity metrics (i.e., λ_1, λ_{23}, and MD) generated more sensitive results than FA [5].

To give a comprehensive view of the degeneration patterns of the white matter in AD and MCI patients, we used the newly developed tract-based spatial statistics (TBSS) method and multiple diffusion indices (FA, MD, λ_1 and λ_{23}) to systematically study AD- and MCI-associated changes in white matter tracts across the whole brain. The TBSS method is a fully automated whole-brain analysis technique that uses voxel-wise statistics on diffusion indices but simultaneously minimizes the effects of misalignment using a conventional voxel-based analysis method [25]. For voxel-based DTI analysis in degenerative diseases such as AD and MCI, atrophy will lead to systematic misalignment to the template for the patients. To circumvent this problem, the TBSS method extracts each subject's white matter skeleton (i.e., the center of all major tracts "common" to all subjects) from the normalized FA images, minimizing the effect of atrophy-induced misregistration. In recent years, TBSS has been widely used to study FA changes in cerebral white matter in AD and MCI patients [5, 20, 26–31]. Although a few studies have investigated alterations of other diffusion metrics (i.e., axial, radial and mean diffusivities) across the brain in AD and MCI, the results were under debate (we will return this issue in Discussion section) [5, 20, 30, 31]. Therefore, a comprehensive analysis of multiple diffusion indices for describing the white matter degeneration patterns in AD and MCI patients was necessary.

MATERIAL AND METHODS

Participants

This study included 52 right-handed subjects (16 AD patients, 17 MCI patients and 19 healthy elderly controls) who gave written informed consent. The AD and MCI patients were recruited from those who had consulted a memory clinic for memory complaints at Xuanwu Hospital, Beijing, China. The healthy elderly controls (HC) were recruited from the local community by advertisements. This study was approved by the Medical Research Ethics Committee of Xuanwu Hospital.

The diagnosis of AD fulfilled the Diagnostic and Statistical Manual of Mental Disorders 4th Edition criteria for dementia [32] and the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer Disease and Related Disorders Association (NINCDS-ADRDA) criteria for possible or probable AD [1]. The subjects were also assessed using the Clinical Dementia Rating (CDR) score [33] (6 patients with CDR = 1 and 10 patients with CDR = 0.5).

Participants with MCI had memory impairment but did not meet the criteria for dementia. The criteria used for the identification and classification of subjects with MCI [34] were the following: (1) impaired memory performance on a normalized objective verbal memory delayed-recall test; (2) a recent history of symptomatic worsening in memory; (3) normal or near-normal performance on global cognitive tests, including a Mini-Mental State Examination (MMSE) score >24, and on activities described in a daily living scale; (4) a global rating of 0.5 on the CDR Scale, with a score of at least 0.5 on the memory domain; and (5) the absence of dementia. All MCI subjects are amnestic MCI (aMCI). The inclusion criteria for the healthy elderly controls were as follows: (1) no neurological or psychiatric disorders such as stroke, depression, or epilepsy; (2) no neurological deficiencies such as visual or hearing loss; (3) no abnormal findings such as infarction or focal lesions on conventional brain magnetic resonance imaging; (4) no cognitive complaints; (5) an MMSE score of 28 or higher; and (6) a CDR score of 0. Notably, the AD and MCI patients exhibited extensive periventricular or deep white matter hyperintensities (WMHs). Here, the presences of WMHs were not considered as exclusive criteria for the MCI and AD patients.

Clinical and demographic data for the participants are shown in Table 1 . There were no significant differences among the three groups in terms of gender, age, or years of education, but the MMSE scores were significantly different ($P < 0.01$) among the groups.

Image acquisition

DTI was performed using a 3.0 T Siemens Trio MR system with a standard head coil. Head motion was minimized with restraining foam pads provided by the manufacturer. Diffusion-weighted images were acquired using a single-shot echo planar imaging (EPI) sequence. An Integral Parallel Acquisition Technique (iPAT) was used with an accelerate factor of 2 as acquisition time and image distortion from susceptibility artifacts can be reduced by the iPAT method. The diffusion sensitizing gradients were applied along 12 non-linear directions ($b = 1000\ \mathrm{s/mm^2}$), together with an acquisition without diffusion weighting ($b = 0\ \mathrm{s/mm^2}$) (average = 4). The imaging parameters were 30 continuous axial slices with a slice thickness of 5 mm and no gap, FOV = 256 mm × 256 mm, TR/TE = 6000/85 ms, and acquisition matrix = 128 × 128. The reconstruction matrix was 256 × 256, resulting in an in-plane resolution of 1 mm × 1 mm.

Table 1
Characteristics of the AD and MCI patients and healthy controls

Characteristics	AD	MCI	Controls	*P* values
N (M/F)	16 (9/7)	17 (8/9)	19 (7/12)	0.52[a]
Age (years)	72.6 ± 6.3	71.5 ± 6.7	69.9 ± 6.2	0.45[*]
Education (years)	10.1 ± 3.4	9.9 ± 3.5	10.2 ± 4.0	0.98[*]
MMSE	18.7 ± 3.1	26.5 ± 1.0	28.6 ± 0.7	<0.01[*]

MMSE: Mini-Mental Status Examination; Plus-minus values are mean ± S.D.

[a]The *P* value for gender distribution in the three groups was obtained using a Chi-square test.

[*]The *P* values were obtained by one-way analysis of variance tests.

Data preprocessing

Three steps were undertaken during preprocessing. First, eddy current distortions and motion artifacts in the DTI dataset were corrected by applying affine alignment of each diffusion-weighted image to the $b = 0$ image using FMRIB's Diffusion Toolbox (FDT) (FSL 4.1.4; www.fmrib.ox.ac.uk/fsl). The first volume of the diffusion data without a gradient applied (i.e., the $b = 0$ image) was then used to generate a binary brain mask using the Brain Extraction Tool. DTIfit was used to independently fit the diffusion tensor to each voxel. The output of DTIfit yielded voxel-wise maps of FA, MD, axial diffusivity (λ_1) and radial diffusivity (λ_{23}) for each subject. Finally, the FA, MD and λ_{23} of each voxel were calculated according to the following formulas:

$$FA = \frac{\sqrt{(\lambda_1 - \lambda_2)^2 + (\lambda_1 - \lambda_3)^2 + (\lambda_2 - \lambda_3)^2}}{\sqrt{2(\lambda_1^2 + \lambda_2^2 + \lambda_3^2)}}$$

$$MD = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3}$$

$$\lambda_{23} = \frac{\lambda_2 + \lambda_3}{2}$$

Tract-based spatial statistics (TBSS)

Tract-based spatial statistics of FA, MD, λ_1 and λ_{23} images were carried out using TBSS in the FMRIB software library (FSL 4.1.4; www.fmrib.ox.ac.uk/fsl; for a detailed description of the methods, see [25]). The steps of the TBSS analyses in our study were as follows:

1. The FA image of each subject was aligned to a pre-identified target FA image (FMRIB58_FA)

by non-linear registrations.
2. All of the aligned FA images were transformed into the MNI152 template (1 mm × 1 mm × 1 mm) by affine registrations.
3. The mean FA image and its skeleton (mean FA skeleton) were created from all subjects.
4. Individual subjects' FA images were projected onto the skeleton.
5. Voxel-wise statistics across subjects were calculated for each point on the common skeleton.

Data for MD, λ_1 and λ_{23} were also generated by applying the above FA transformations to the additional diffusivity maps and projecting them onto the skeleton using identical projection vectors to those inferred from the original FA data.

Statistical analyses

We first calculated the mean diffusion indices (FA, MD, λ_1 and λ_{23}) in the whole-brain white matter skeleton for each subject. We then performed two-sample *t*-tests to compare the mean diffusion indices between any two groups: MCI versus HC, early AD versus HC, and early AD versus MCI.

Voxel-wise statistics in TBSS were carried out using a permutation-based inference tool for nonparametric statistical thresholding ("randomize," part of FSL, see [25]). In this study, voxel-wise group comparisons were performed using non-parametric, two-sample *t*-tests in: MCI versus HC, early AD versus HC, and early AD versus MCI. The mean FA skeleton was used as a mask (thresholded at a mean FA value of 0.2), and the number of permutations was set to 5,000. The significance threshold for between-group differences was set at $P < 0.05$ (FWE corrected for multiple comparisons) using the threshold-free cluster enhancement (TFCE) option in the "randomize" permutation-testing tool in FSL [35].

RESULTS

Mean diffusion indices of white matter skeletons

Two-sample *t*-tests showed that the AD patients had significantly lower fractional anisotropy (FA, $P = 0.02$), higher mean diffusivity (MD, $P = 0.002$), higher axial diffusivity (λ_1, $P = 0.02$) and higher radial diffusivity, (λ_{23}, $P = 0.002$) in their white matter skeletons than the healthy controls. In MCI no significant differences were observed for any diffusion indices with neither AD nor healthy controls ($P < 0.05$) (Table 2).

TBSS analyses between groups

Early AD versus HC

The TBSS analyses revealed that the AD patients had significantly reduced FA in widespread brain regions compared with the controls, including the white matter of the lateral temporo-parietal regions, the posterior cingulate cortex/precuneus (PCC/PCu), the fronto-parietal regions and the whole corpus callosum (Fig. 1). The results for increased diffusivities (MD, λ_1 and λ_{23}) were broadly concordant with those for reduced FA. The radial (λ_{23}) and mean diffusivity (MD) changes were slightly more extensive than those for axial diffusivity (λ_1). There were no white matter tracts that showed increased FA or decreased diffusivities (MD, λ_1 and λ_{23}) in the AD patients compared to the controls.

MCI versus HC

The MCI subjects had significantly reduced FA in the white matter of the PCC/PCu and inferior frontal cortex in the left hemisphere compared to the controls (Fig. 2). There were no white matter tracts that showed increased FA in the MCI patients. There were also no significant differences in the absolute diffusivities (MD, λ_1 and λ_{23}) between MCI and HC groups.

Table 2
Mean diffusion indices of white matter skeletons for each group

	AD	MCI	HC	*P* values		
				AD vs. HC	MCI vs. HC	AD vs. MCI
FA	0.39 ± 0.02	0.40 ± 0.02	0.41 ± 0.02	0.023^*	0.11	0.48
MD ($\times 10^{-3}$ mm^2/s)	0.81 ± 0.04	0.79 ± 0.03	0.78 ± 0.04	0.002^*	0.13	0.053
λ_1 ($\times 10^{-3}$ mm^2/s)	1.17 ± 0.04	1.15 ± 0.03	1.14 ± 0.04	0.011^*	0.37	0.067
λ_{23} ($\times 10^{-3}$ mm^2/s)	0.63 ± 0.04	0.60 ± 0.03	0.58 ± 0.04	0.002^*	0.094	0.072

*Significant group differences at $P < 0.05$.

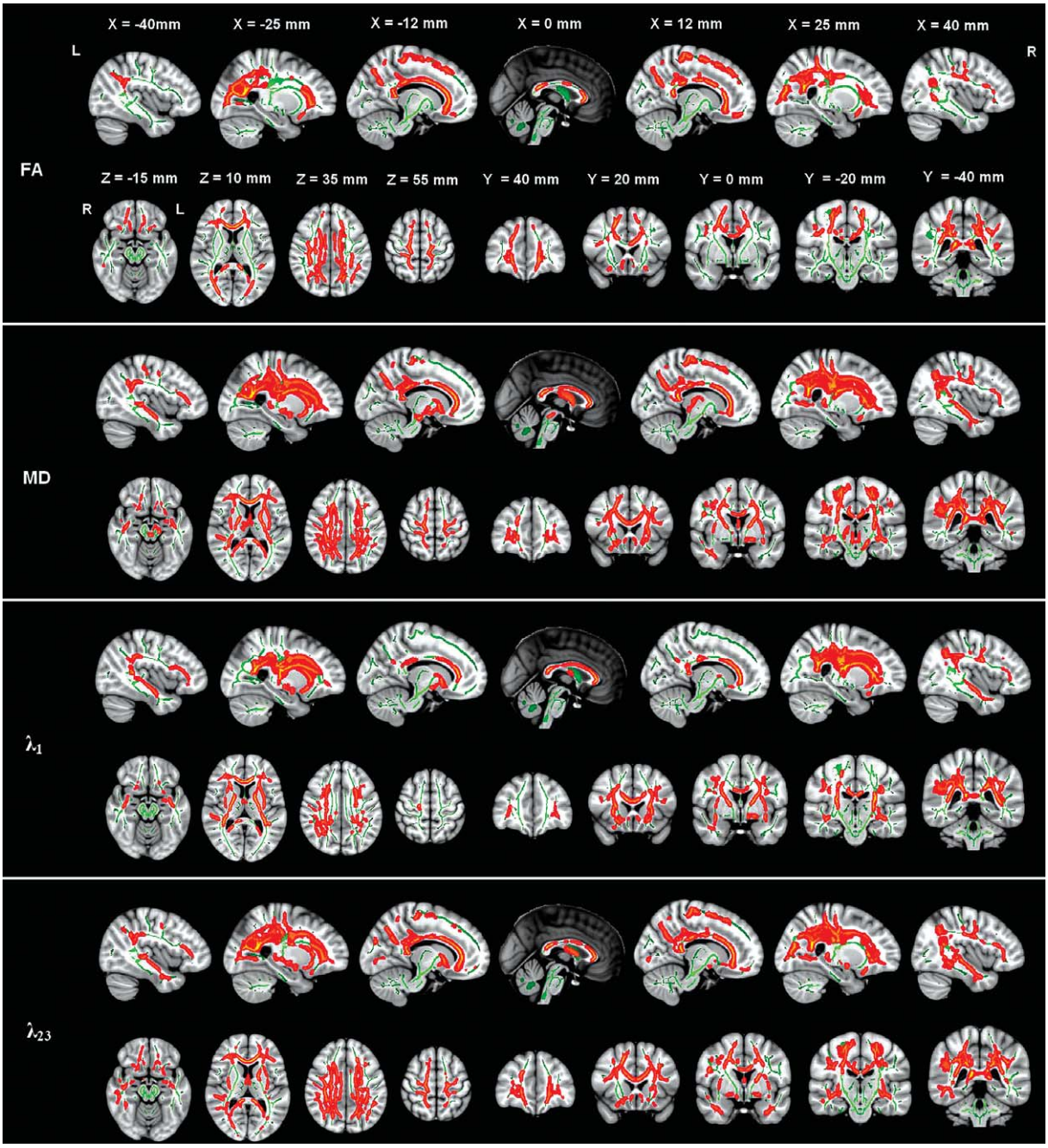

Fig. 1. TBSS results of the diffusion indices between the AD and HC groups. Green represents the mean white matter skeleton of all subjects; red represents the regions with reduced FA (1st row), increased MD (2nd row), increased λ_1 (3rd row) and increased λ_{23} (4th row) in the early AD patients. The TBSS results for increased FA or decreased diffusivities (MD, λ_1 and λ_{23}) in the early AD patients did not show any statistically significant differences at $P < 0.05$ (FWE corrected for multiple comparisons).

Early AD versus MCI

There were no significant differences found in FA between the AD and MCI patients; however, we observed that the AD patients had increased MD in bilateral temporo-parietal regions, bilateral PCC/PCu, and the genu and splenium of the corpus callosum com-

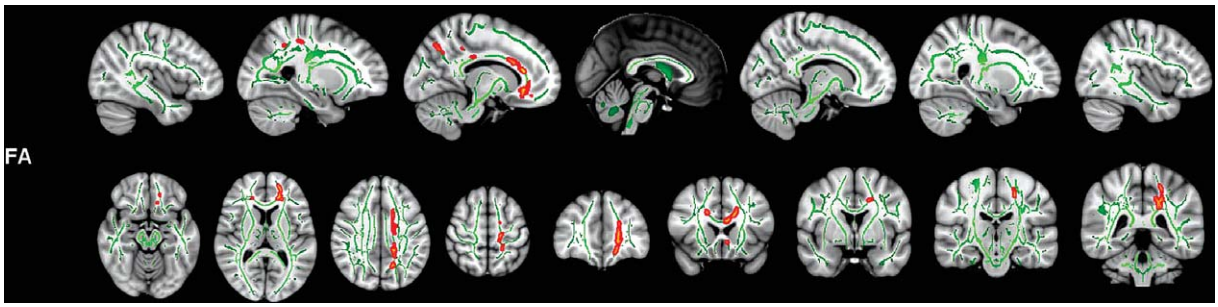

Fig. 2. TBSS results of the diffusion indices between the MCI and HC groups. Green represents the mean white matter skeleton of all subjects; red represents the regions with reduced FA in the MCI subjects. The TBSS results for increased FA or increased/decreased diffusivities (MD, λ_1 and λ_{23}) in the MCI subjects did not show any statistically significant differences at $P<0.05$ (FWE corrected for multiple comparisons).

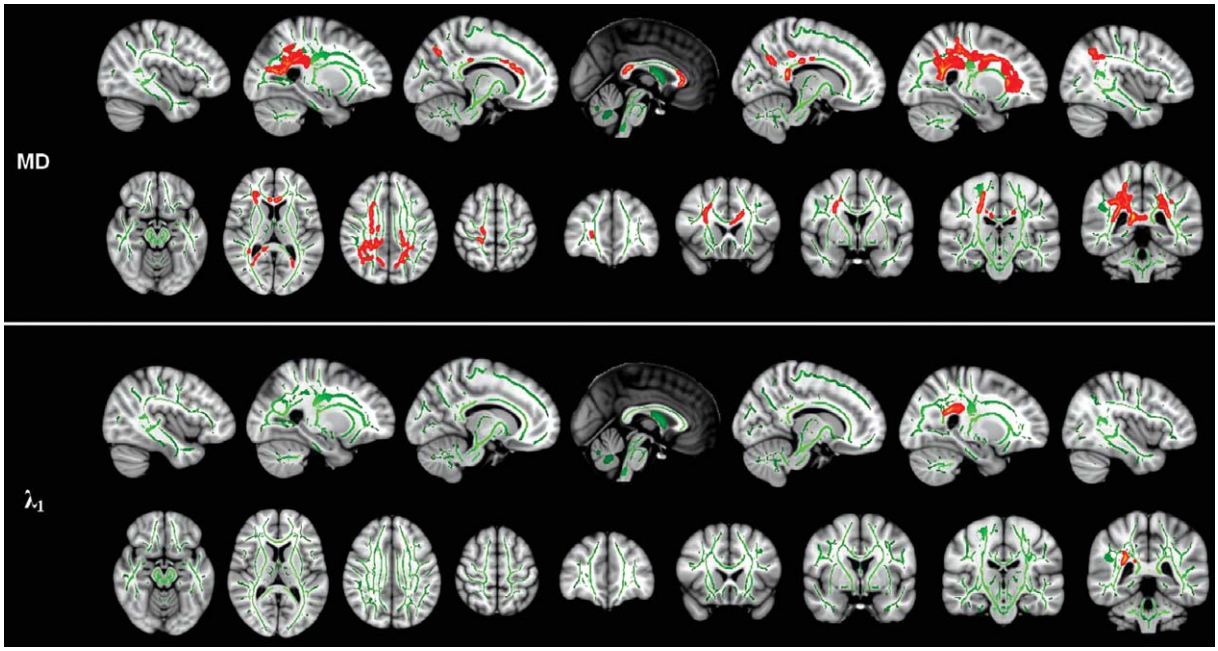

Fig. 3. TBSS results of the diffusion indices between the AD and MCI groups. Green represents the mean white matter skeleton of all subjects; red represents the regions with increased MD and increased λ_1 in the early AD patients. The TBSS results for increased/decreased FA or decreased diffusivities (MD, λ_1 and λ_{23}) in the early AD patients did not show any statistically significant differences at $P<0.05$ (FWE corrected for multiple comparisons).

pared to the MCI patients (Fig. 3). Only one cluster located in the white matter adjacent to the PCC/PCu showed increased axial diffusivity (λ_1) in the AD patients. There were no significant differences in the radial diffusivities (λ_{23}) between AD and MCI groups.

DISCUSSION

In this study, we determined global maps of the white matter changes in AD and MCI patients by measuring fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (λ_1) and radial diffusivity (λ_{23}) across the brain, offering a comprehensive view of the landscape of white matter degeneration in AD and MCI. Compared with the healthy elderly controls, the AD patients exhibited significantly reduced FA and increased axial, radial and mean diffusivities in widespread white matter regions. Some regions with reduced FA were observed in the MCI patients as compared to the controls. Moreover, between the AD and

MCI groups, there were significant differences in the diffusivity metrics (i.e., MD and λ_1) in several white matter regions without FA changes. Taken together, these data provide empirical evidence for degenerative changes in widespread white matter structures in AD and MCI patients. The findings suggest distinct degeneration behaviors of the white matter in AD and MCI.

Distributed white matter regions with diffusion changes in AD and MCI

AD versus HC

We identified AD-related abnormalities in widespread white matter regions, including lateral temporo-parietal regions, PCC/PCu and the fronto-parietal cortex. And reduced FA involved most of the white matter pathways, including bilateral superior longitudinal fasciculus, inferior longitudinal fasciculus, uncinate fasciculus, cingulum bundles, corticospinal tracts and corpus callosum. Most of these affected tracts have been reported in previous DTI studies in AD using regions of interest (ROI)-based or conventional voxel-based methods [16, 24, 36–39] and were found to be related to the cognitive dysfunctions in patients with AD [27, 30, 40, 41]. Furthermore, our finding are also parallel to the findings from recent TBSS studies in AD [5, 20, 26–30]. Specifically, one TBSS study reported diffusion abnormalities in the "default-mode" network (DMN)-related white matter structures, particularly in posterior components including the PCC/PCu, lateral temporo-parietal regions and the splenium of corpus callosum [5]. Another TBSS study found decreased FA only in the left temporal lobe [28]. Several other studies revealed FA reduction in many white matter tracts, such as uncinate fasciculus, superior longitudinal fasciculus, inferior longitudinal fasciculus, cingulum bundles and corpus callosum [20, 26, 27, 30]. The differences between the results of these TBSS studies could be due to different samples of AD or imaging parameters employed. However, it is worthy to note that they have consistently demonstrated widespread abnormalities of many white matter regions in AD patients, especially in the posterior areas.

MCI versus HC

In the MCI patients, we found that the regions with reduced FA were mainly located in the white matter of the PCC/PCu and inferior frontal cortex in the left hemisphere, to a less extent than in AD patients. For MCI subjects, the structural and functional abnormalities in the posteromedial cortical circuit (e.g., the medial temporal lobe and PCC/PCu), which represents the prodromal changes of AD, have been consistently reported [3, 42–44]. Several DTI studies also demonstrated that the posterior cingulum, especially on the left, were affected in MCI when compared to controls [16, 39, 45], which provides support for our asymmetric findings. Considering recent TBSS studies in MCI [26–28, 30], the discrepancies among different studies need to be paid attention. First, two TBSS studies did not show significant differences in FA between MCI and healthy controls [28, 30]. In contrast, Serra and colleagues found that MCI patients had reduced FA in the anterior part of the right anterior thalamic radiation [27]. Additionally, Liu and colleague found decreased FA in the right parahippocampal white matter, bilateral uncinate fasciculus and tracts in the brain stem and cerebellum with an uncorrected threshold [26]. In our study, the white matter regions with FA changes in MCI are located in the PCC/PCu and inferior frontal cortex of the left hemisphere. The discrepancies between these studies could be attributable to different data samples, imaging parameters or the heterogeneous groups of subjects (converters and non-converters).

AD versus MCI

Between the AD and MCI groups, the regions with the most significant differences were located in the white matter adjacent to the PCC/PCu, which is a critical node in the human brain networks [46–48]. Many studies have consistently demonstrated that this region shows structural and functional abnormalities in AD and MCI patients [42, 49–53]. Moreover, between MCI and AD patients structural and functional differences in the PCC have been also reported [16, 26, 39, 54]. A DTI study suggested that the fractional anisotropy of the PCC can significantly improve the distinction of MCI and AD patients from healthy elderly subjects [39]. Taken together, our results imply that the PCC/PCu is a key region affected by AD and MCI, suggesting that the structural characteristics of this region could be useful for early detection and monitoring of disease progression. Furthermore, we found that no regions showed significant differences in FA between AD and MCI, which was consistent with the findings of two previous TBSS studies [28, 30].

Neural substrates of diffusion changes

In this study, we used four diffusion indices (FA, MD, λ_1 and λ_{23}) to investigate diffusion changes in

the white matter in AD and MCI patients, generating a comprehensive view of the landscape of white matter tract degeneration. Compared with the healthy controls, the AD patients showed significantly reduced FA and increased MD, λ_1 and λ_{23} in many brain regions, consistent with the findings of recent TBSS studies in AD [5, 30]. These changes in diffusion indices may underlie micro-structural changes in the white matter. Many studies on neurological diseases and disorders have observed regional reductions in anisotropy, and some researchers have proposed that the primary determinant of anisotropy is the packing density of axons within a voxel [12, 55]. Axonal packing density encompasses a variety of microstructural level variables (e.g., degree of myelination, axonal diameters, and extracellular space). Therefore, similar FA reductions may not be interpreted in the same way depending on the changes in the individual eigenvalues. Decreased axial diffusivity may reflect axonal loss or damage [21, 22, 56] and increased radial diffusivity may suggest demyelination and a loss of myelin integrity [19, 57]. More severe decreases in axonal packing density (e.g., from a greater loss of myelin or axons) would lead to a global increase in extracellular water, resulting in larger radial diffusivity increases and subsequent axial diffusivity increases [5, 18].

In AD patients, we found some regions with increased radial diffusivity, but no changes in axial diffusivity, such as white matter of the frontoparietal regions (superior longitudinal fasciculus) and PCC/PCu (cingulum bundles). This pattern of diffusion changes has been reported by several recent TBSS studies in AD, normal aging and multiple sclerosis [18, 26, 30, 58]. In animal literatures the changes in radial diffusivity is related to myelin deficient or myelin loss [19, 57]. Thus, the abnormalities in white matter observed here might reflect alterations of integrity of myelin, which was in accordance with retrogenesis through mechanisms outlined by Bartzokis et al. [59]. On the other hand, some regions were found to have increased axial diffusivity, but no changes in radial diffusivity, such as the internal capsule and some regions around the cerebral ventricles. The interpretation of axial diffusivity variations is controversial in pathological conditions because both increases and decreases have been reported [5, 18, 30]. The underlying mechanisms may be related to axonal damages and fiber re-organization. In the present study, we noticed that AD and MCI patients exhibited a more extensive periventricular WMHs compared to controls. The findings of increased axial diffusivity, without changes in radial diffusivity around the cerebral ventricles are possibly related to the occurrence of WMHs in the patients group. Additionally, other regions showed isotropic changes in axial and radial diffusivities, including white matter of the lateral temporal cortex and the corpus callosum. According to the previous studies, we speculated that these increases in both axial and radial diffusivity may be due to atrophy of the gray matter and an increase in the extracellular space, which could be due to a greater loss of myelin or axons from neurodegenerative processes or microvascular pathology [10]. However, it should be noted that the correspondence between axon and myelin damages and tensor diffusivities is still controversial with the current resolution of DTI sequences. Thus, we can not resolve histopathological implications in axial versus radial diffusivity changes and clinical correlations in the diseases.

Compared to FA, more sensitive results were obtained by using absolute diffusivity metrics (MD, λ_1 and λ_{23}) in the AD patients. For example, we identified the white matter of the lateral temporal cortex as having increased axial, radial and mean diffusivities, but no changes in FA. FA was inherently less sensitive in this situation because the axial and radial diffusivities changed in the same direction. Therefore, the analysis of component eigenvalues were necessary to capture the full extent of white matter changes [5]. Furthermore, the biological processes were not the same across different stages of the disease. We found that the most significant change between the healthy elderly and MCI groups was decreased FA in the white matter of the left hemisphere, while the most significant changes between the MCI and AD groups were increased axial and mean diffusivities in posterior white matter regions. Differences in the effects on glia, mechanisms of axonal degeneration, inflammatory responses and even the rate of degeneration could all give rise to different tensor behavior in different disease stages. Therefore, exploring the component eigenvalues of the tensor would be advantageous in studying all degenerative diseases rather than assuming that a single metric (such as FA) is sufficiently sensitive to different pathological states.

Methodological issues

Several methodological issues need to be addressed. First, we used a 5-mm slice thickness for the DTI data. In the TBSS analyses, the DTI data were converted into MNI space with an isotropic resolution of

1 mm × 1 mm × 1 mm. A previous study has shown that the non-isotropic voxel dimensions can underestimate the FA in areas with crossing fibers [60]. It may additionally confound the calculation of all diffusion indices, especially given the procedure for TBSS to reslice the low resolution voxels to 1 mm isotropic data in the present study. Therefore, diffusion changes less than 5 mm in the z-axis direction cannot be reliably evaluated due to the resolution limit, especially when evaluating small tracts such as the fornix. Based on the results of this study, all of the clusters with diffusion changes in the early AD and MCI patients were more than 5 mm in length, and this might minimize the limitations of slice thickness. To deal with the "fiber crossing" problem, several recent studies have proposed advanced imaging techniques, such as diffusion spectral imaging [61, 62] or high angular resolution diffusion imaging with Q-ball reconstruction of multiple fiber orientations [63, 64]. Therefore, future studies with improved data quality and advanced imaging techniques are needed to further validate our findings. Second, in this cross-sectional DTI study, all MCI participants were identified with aMCI according to the criteria: an isolated memory impairment with no neuropsychological evidence of any additional cognitive deficits. According to Petersen's criteria [65], these features may be considered as a prodromal state of AD. A large body of literature indicates that this subtype tends to progress to AD at a rate of 10% to 15% per year [34]. Thus, the longitudinal follow-up study would be important to identify the aMCI subjects who will convert to AD. It would be helpful to evaluate the preclinical abnormalities in AD and explore the changing patterns of white matter between converters and non-converters.

In summary, by using TBSS analyses, we generated a global map of the white matter changes in AD and MCI patients by measuring anisotropy and diffusivity changes across the brain. Compared with healthy elderly controls, the AD patients showed significantly reduced anisotropy and increased axial, radial and mean diffusivities in distributed white matter regions, particularly in the white matter of the posterior cingulate cortex/precuneus, lateral temporal cortex and the fronto-parietal cortex, in addition to the interhemispheric connections. In MCI, we found white matter changes in similar regions but with much less extent than in AD. Moreover, our findings suggest distinct degeneration behaviors of the white matter in AD and MCI. In future, the follow-up studies are necessary to determine the clinical values of the various DTI indices to predict AD or longitudinal DTI measurements.

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